

Response to Reviewer Comment 1 | EGUSPHERE-2023-2097

“Long-term legacy of phytoremediation on plant succession and soil microbial communities in petroleum-contaminated sub-Arctic soils”

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*We would like to thank the reviewer for their thoughtful feedback and comments on our manuscript. The reviewer identified several important points that we have addressed which will increase the accessibility and reproducibility of this study. The original comments are written in black and our reply in **blue and italics**.*

The manuscript investigates into a reevaluation of a petroleum-contaminated site in Fairbanks, Alaska, fifteen years after the introduction of annual grasses and/or fertilizer treatments to investigate their impact on the degradation of petroleum hydrocarbons. The study meticulously examines contaminant concentrations, as well as the dynamics of plant and soil microbial ecology. This research is particularly significant given the infrequency of long-term phytoremediation studies, acknowledging the necessity for extended investigations in a field where remediation timelines often exceed those of conventional physico-chemical treatments.

The overall quality of the study is commendable, featuring an ample number of figures, tables, and well-referenced content.

I propose the following suggestions to enhance the manuscript:

1. **Script Accessibility:** It would be beneficial if the authors could provide a link to the scripts employed for statistical and sequencing analyses on a platform such as GitHub. This transparency enhances the reproducibility of the study.

We appreciate this feedback and agree that it is important to increase the transparency and accessibility of data and associated coding. To this end, we have added the scripts to a github repository (github.com/mcleewis/FarmersLoop) and added the link and coding accessibility statement to the methods section (line 185 and 205, in the revised manuscript).

2. **Pyrosequencing Data Clarification:** The authors are encouraged to include a statement elucidating the comparability between results obtained through pyrosequencing and Next-Generation Sequencing (NGS). A justification for the relevance of publishing pyrosequencing data in the context of modern microbiome studies would further strengthen the manuscript.

We appreciate this feedback that pyrosequencing is an outdated technology however when the original study was conducting, the sequencing was the appropriate technology. While it has

taken a long time for this paper to reach review, community assessment by pyrosequencing still has value as a tool. In several studies that explore the two technologies with the same samples, the abundance of genes, genotypes, and communities were found to be highly correlated ($R>0.9$) (for example: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0030087> and others). To acknowledge the shortcoming associated with pyrosequencing, we have added a statement to the methods section (line 174-176, in the revised manuscript).

3. Enhanced Figure Visualization: Figures resembling the color-coded schemes in Figures 3 and 4 would significantly enhance the clarity of the manuscript. The use of color could aid in distinguishing between different treatments, facilitating a more accessible interpretation of the data.

Thank you for this comment, while the original figures were built in greyscale to ensure that the figures are readable by any with potentially colour blindness, we agree that they benefit from the addition of colours. We have re-made most figures (i.e., Figure 4, 5, S1, S2, S3, S5) to include both colour and changes in shape.

4. Comparison of Concentrations: While the manuscript notes that crude oil and diesel concentrations remain around 500 ppm after a decade of treatment, it would be valuable to compare and discuss these findings with other bioremediation and phytoremediation studies. An exploration of whether similar concentrations are observed in other studies after a decade of treatment would contribute valuable insights to the field, as those are expected to be lower.

Thank you for this comment, we have changed the way the TPH data are presented (Table S2) to highlight the overall loss more clearly over the course of all the measured time points. In addition, we added to discussion (line 456-457, in the revised manuscript), to link the percent disappearance presented here to other phytoremediation studies conducted in Northern areas.